A Protein–Leucine Supplement Increases Branched-Chain Amino Acid and Nitrogen Turnover But Not Performance

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[1]School of Sport and Exercise, Massey University, Wellington, NEW ZEALAND; [2]Department of Kinesiology, McMaster University, Hamilton, Ontario, CANADA; [3]Nestle Research Center, Lausanne, SWITZERLAND; and [4]School of Health and Human Sciences, Southern Cross University, Lismore, New South Wales, AUSTRALIA

ABSTRACT

NELSON, A. R., S. M. PHILLIPS, T. STELLINGWERFF, S. REZZI, S. J. BRUCE, I. BRETON, A. THORIMBERT, P. A. GUY, J. CLARKE, S. BROADBENT, and D. S. ROWLANDS. A Protein–Leucine Supplement Increases Branched-Chain Amino Acid and Nitrogen Turnover But Not Performance. *Med. Sci. Sports Exerc.*, Vol. 44, No. 1, pp. 57–68, 2012. **Purpose**: This study aimed to determine the effect of postexercise protein–leucine coingestion with CHO–lipid on subsequent high-intensity endurance performance and to investigate candidate mechanisms using stable isotope methods and metabolomics. **Methods**: In this double-blind, randomized, crossover study, 12 male cyclists ingested a leucine/protein/CHO/fat supplement (LEUPRO 7.5/20/89/22 $g \cdot h^{-1}$, respectively) or isocaloric CHO/fat control (119/22 $g \cdot h^{-1}$) 1–3 h after exercise during a 6-d training block (intense intervals, recovery, repeated-sprint performance rides). Daily protein intake was clamped at 1.9 $g \cdot kg^{-1} \cdot d^{-1}$ (LEUPRO) and 1.5 $g \cdot kg^{-1} \cdot d^{-1}$ (control). Stable isotope infusions (1-^{13}C-leucine and 6,6-$^{2}H_2$-glucose), mass spectrometry–based metabolomics, and nitrogen balance methods were used to determine the effects of LEUPRO on whole-body branched-chain amino acid (BCAA) and glucose metabolism and protein turnover. **Results**: After exercise, LEUPRO increased BCAA levels in plasma (2.6-fold; 90% confidence limits = ×/÷1.1) and urine (2.8-fold; ×/÷1.2) and increased products of BCAA metabolism plasma acylcarnitine C5 (3.0-fold; ×/÷0.9) and urinary leucine (3.6-fold; ×/÷1.3) and β-aminoisobutyrate (3.4-fold; ×/÷1.4), indicating that ingesting ~10 g leucine per hour during recovery exceeds the capacity to metabolize BCAA. Furthermore, LEUPRO increased leucine oxidation (5.6-fold; ×/÷1.1) and nonoxidative disposal (4.8-fold; ×/÷1.1) and left leucine balance positive relative to control. With the exception of day 1 (LEUPRO = 17 ± 20 mg $N \cdot kg^{-1}$, control = −90 ± 44 mg $N \cdot kg^{-1}$), subsequent (days 2–5) nitrogen balance was positive for both conditions (LEUPRO = 130 ± 110 mg $N \cdot kg^{-1}$, control = 111 ± 86 mg $N \cdot kg^{-1}$). Compared with control feeding, LEUPRO lowered the serum creatine kinase concentration by 21%–25% (90% confidence limits = ±14%), but the effect on sprint power was trivial (day 4 = 0.4% ± 1.0%, day 6 = −0.3% ± 1.0%). **Conclusions**: Postexercise protein–leucine supplementation saturates BCAA metabolism and attenuates tissue damage, but effects on subsequent intense endurance performance may be inconsequential under conditions of positive daily nitrogen balance.
Key Words: STABLE ISOTOPE, ENDURANCE, NITROGEN BALANCE, METABOLOMICS, MEMBRANE DAMAGE

Endurance athletes frequently undertake high-intensity exercise on consecutive days, such as during training camps or multiday competition. Such exercise depletes glycogen stores (19) and can disrupt skeletal muscle structural integrity (20), but it is also an adaptive stimulus (14,33). Nutrition plays an important role in the restoration of muscle glycogen, and emerging evidence indicates a role for dietary protein and amino acids in attenuating skeletal muscle damage and increasing muscle protein turnover to promote adaptive remodeling (14,17). For these reasons, there is considerable interest in the role of postexercise protein, amino acid, and CHO ingestion in the mechanisms associated with recovery and training adaptation.

Evidence for an ergogenic effect of postexercise protein-rich food on subsequent endurance performance was reported recently (32,34,37). Only limited mechanistic investigation was undertaken, but the effect was associated with positive nitrogen balance and attenuation of plasma creatine kinase (CK) concentrations. Other evidence points to a postexercise protein nutrition–mediated effect via adaptive remodeling of structural and contractile elements in the exercised skeletal muscle (33), supported by an enhanced fractional protein synthesis rate (FSR) (17).

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Recently, our group revealed that feeding well-trained cyclists milk proteins (4 × 15 g·30 min^{-1} for 1.5 h) with additional free leucine (4 × 3.75 g·30 min^{-1}), CHO, and lipid after intense endurance cycling daily for 3 d resulted in a small improvement in repeated-sprint mean power 2 d later, relative to an isocaloric low-protein–lipid control (37). Leucine is of special interest because it stimulates muscle protein synthesis (2,7) and inhibits protein degradation (27), although some evidence suggests that leucine ingested at rest without recent exercise at doses near the maximum tissue oxidative capacity (estimated at ~40 g·d^{-1} [9]) could be excessive and less useful to muscle protein metabolism when consumed out of proportion to valine and isoleucine (13). With evidence for a functional effect, the objective of the present study was not only to provide additional end point phenotype information on the effect of a postexercise protein–leucine-rich supplement on subsequent performance but to also investigate putative metabolic mechanisms that might explain the performance effect. Possible alterations to CHO metabolism warranted further investigation, as did the effect of protein–leucine supplementation on BCAA metabolism.

To investigate these questions, we used a whole-body stable isotope method to study leucine and glucose turnover, and contemporary mass spectrometry–based metabolomics to assess large-scale changes in metabolites in response to postexercise protein–leucine ingestion. We also sought to confirm the benefit of the leucine-rich supplement on subsequent high-intensity endurance cycling performance several times throughout a 6-d block of controlled training and diet. Our hypothesis was that the leucine-enriched protein recovery supplement would increase BCAA metabolism and nonoxidative disposal and enhance subsequent high-intensity endurance cycling performance.

METHODS

Participants

Twelve well-trained male cyclists (mean ± SD: age = 35 ± 10 yr, height = 182 ± 5 cm, body mass = 76.9 ± 6.5 kg), with a maximal oxygen uptake ($\dot{V}O_{2max}$) of 64.8 ± 6.8 mL·kg^{-1}·min^{-1} and peak power output (W_{max}) of 355 ± 36 W, completed the study. Cyclists had a 9 ± 4-yr training history, with a recent weekly training volume of 10 ± 1 h. Participants were screened for contraindications and excluded on the basis of the following: failed to pass a health screening; recently donated blood; recently consumed caffeine, alcohol, medications/drugs; or smoked during the experimentally controlled period. All participants were informed of the purpose of the study and associated risks and provided written informed consent. The study was approved by the Central Regional Ethics Committee of New Zealand.

General Design

This is a double-blind, randomized, crossover study to determine the effect of a leucine + protein–CHO–fat (LEUPRO) postexercise feeding intervention compared with an isocaloric CHO–fat control on repeated-sprint cycling performance and mechanistic variables during a 6-d block of controlled high-intensity training and diet (Fig. 1). Before the first experimental block, participants recorded their habitual training and diet for 7 and 3 d, respectively; training

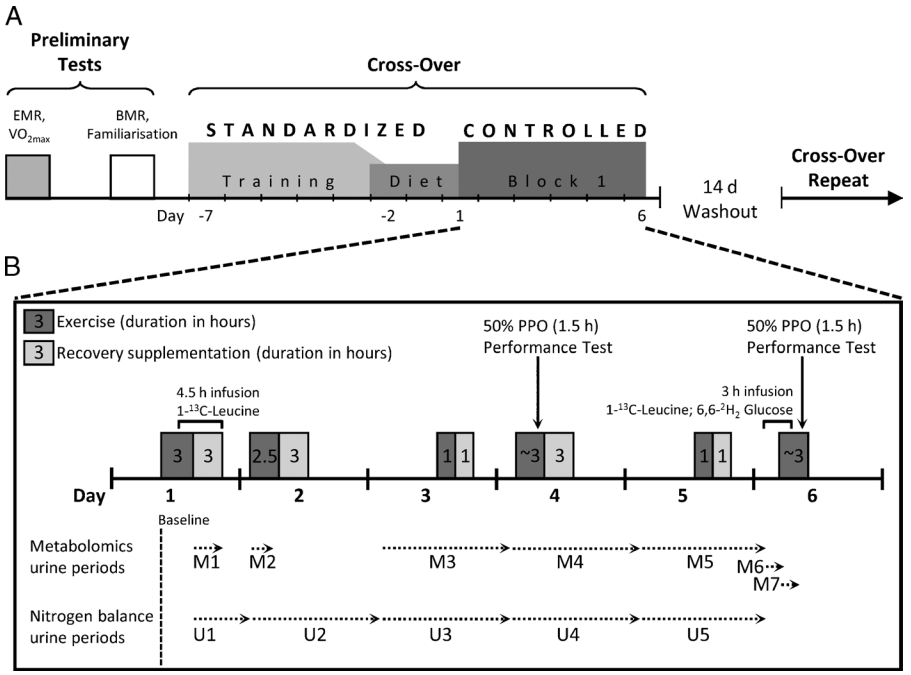

FIGURE 1—Experimental design. Shown are preliminary visits and standardized training and diet leading in to a 6-d experimental block (A), with one experimental block further outlined, including high-intensity cycling, recovery supplementation, and urine collection periods (B). M1–M7, metabolomics urine collection periods 1–7; U1–U5, urine collection periods 1–5.

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was tapered in the 3 d before the experimental block, including a rest day before starting. To standardize lead-in fatigue and minimize diet variation, this training and dietary regimen was repeated preceding block 2.

Preliminary Testing

Two weeks before the first experimental block, cyclists underwent exercise metabolic rate (EMR), $\dot{V}O_{2max}$, and W_{max} tests on an electromagnetically braked cycle ergometer (Velotron, Version 1.9 Software; Racer Mate, Seattle, WA). The exercise protocol consisted of 10 min at 100 W and 3 × 6-min stages at 150, 188, and 225 W (for EMR) after which the workload was increased by 25 W every 2.5 min until volitional exhaustion or failure to maintain a cadence of at least 60 rpm. Expired breath was collected into Douglas bags (VacuMed 1195-200; GBC BioMed, Auckland, New Zealand) during the last half of each increment, then continuously near exhaustion. Subsequent W_{max} was calculated (34), and values used to establish workloads were used during the main experimental protocol. $\dot{V}O_{2max}$ was determined with minute volume measured by a pneumotach (Hans Rudolph, Inc., Kansas City, MO) and oxygen and carbon dioxide analyzers (Vmax Spectra Series; SensorMedics Corp., Yorba Linda, CA). During the three submaximal stages, EMR ($kJ \cdot min^{-1}$) was calculated from oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) rates (18).

One week after, cyclists reported to the laboratory (6:00 a.m.) in fasted condition for calculation of their basal metabolic rate (RMR) via indirect calorimetry (18). Subjects were supine for 20 min followed by 30 min of expired breath collection. Subsequently, a small breakfast was provided before participants engaged in a full familiarization trial of the repeated-sprint performance test as described previously (34). During all laboratory visits, environmental conditions were a temperature of 19.3°C ± 1.4°C and a relative humidity of 42% ± 7%.

Experimental Protocols

Exercise procedures. The riding schedule was a multiday model adopted from that used previously (32) to simulate the physical stress involved in strenuous high-intensity training or competition. Workloads were preprogrammed at fixed percentages of individual W_{max}. Each block comprised four intermittent high-intensity rides. Day 1 (3 h of cycling) consisted of a warm-up (15 min at 30%, 10 min at 40%, and 6 min at 50% W_{max}) followed by loading intervals: three blocks of 10 × 2-min intervals at 90%/80%/70% W_{max} (2 min at 50% W_{max} between intervals and 6 min at 50% W_{max} between blocks) and a cool down of 11 min at 30% W_{max}. Day 2 (2.5 h of cycling) comprised a warm-up (10 min at 30%, 10 min at 40%, 5 min at 50% W_{max}) followed by two blocks of longer loading intervals (4 × 5 min at 70% W_{max} interspersed with 3 × 5 min at 50% W_{max} and 3 × 4 min at 70% interspersed with 3 × 4 min at 50% W_{max}) and separated by 3 × 1 min at 90% and 3 × 1 min at 80% W_{max} (interspersed by 2 min at 50% W_{max}). Days 4 and 6 comprised 90 min at 50% of W_{max} followed by the repeated-sprint performance test as described by Rowlands et al. (32,34). Rides on days 3 and 5 comprised 60 min at 30% of W_{max}. All rides were conducted at the same time of day for a given participant: between 2:00 and 6:00 p.m. for days 1, 3, and 5 and between 5:00 and 9:00 a.m. for days 2, 4, and 6.

Energy expenditure. Total daily energy expenditure (∑EE) for each day of the experimental block was estimated from RMR and EMR and daily activity energy expenditure: ∑EE (kJ) = PA + EMR + TEF, where PA is the sum of daily physical activities, and TEF is the thermogenic effect of food assumed as 10% of expended daily energy plus exercise energy expenditure (i.e., TEF = 0.1[PA + EMR]). To estimate PA, participants prospectively diary-logged all activities during the experimental block, which were converted to METs (1). Uncompleted or extra activities were recorded and repeated during the subsequent experimental block. Energy expenditure during exercise was estimated from the regression of the three submaximal EMR versus workload samples obtained during $\dot{V}O_{2max}$ testing.

Control of diet. To reduce the endogenous ^{13}C breath background enrichment, participants were instructed to avoid foods with CHO sources naturally enriched in ^{13}C (maize, sugar cane, or sugar beet) starting 7 d before the experimental block. All food was provided during the 6-d experimental blocks. Inclusive of recovery supplementation, dietary protein intake was clamped at 1.5 $g \cdot kg^{-1} \cdot d^{-1}$ for control condition, midrange within estimated requirements for endurance training men (35), and 1.9 $g \cdot kg^{-1} \cdot d^{-1}$ for the LEUPRO condition, with the protein difference provided in the postexercise intervention. The daily protein intake for LEUPRO approximates the value estimated to achieve nitrogen balance from our previous 4-d high-intensity cycling protocol (32). The diet provided ≥8 g $CHO \cdot kg^{-1} \cdot d^{-1}$ on heavy training days (days 1, 2, and 4) for both conditions to ensure sufficient glycogen resynthesis (6). Daily energy intake was designed to balance the estimated daily energy expenditure. A balancing supplement comprising milk protein, maltodextrin, and cream powder was provided with evening meals to ensure total daily requirements were met (see Supplementary Digital Content 1, http://links.lww.com/MSS/A109 for mean daily macronutrient and energy intake). Before the morning rides (days 2, 4, and 6), cyclists were provided a small CHO-rich breakfast of toast, butter, and honey to simulate normal practice (50/10/9 g CHO/protein/fat). At 30 min after ingesting the final serving of intervention on days 1, 2, and 4, participants received a small pasta meal (36/7.5/2.0 g CHO/protein/fat). Cyclists ingested a 7.5% CHO sports drink comprising 2:1 maltodextrin to glucose (tapioca and wheat origin, respectively), 1.17 $g \cdot L^{-1}$ NaCl, and lime juice, provided every 30 min during exercise (every 15 min during performance tests) at a rate of 790 ± 82 mL of water per hour.

Nutritional intervention. At postexercise urine collection and blood sampling, cyclists ingested the intervention

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beverages, which comprised two identically flavored milk-like emulsions prepared from dried ingredients. The supplements were prescribed for a model rider with W_{max} of 360W and then scaled to each participant on the basis of W_{max} (grams of leucine or macronutrient multiplied by W_{max}/360 W) so that riders with greater energy expenditures received more nutrition, with a per-serve final intake of 3.8 ± 0.4 g leucine, 10.0 ± 1.0 g protein, 44.0 ± 4.6 g CHO, and 11.0 ± 1.1 g fat for LEUPRO and 60.0 ± 6.2 g CHO and 11.0 ± 1.1 g fat for control into 250 ± 72 mL of water. One serving was provided every 30 min for a total of six servings after exercise on days 1, 2, and 4 and two servings on days 3 and 5. In addition, water was provided, equating to weight lost during exercise. L-Leucine was from Dolder AG (Basel, Switzerland). Whole protein was a micellar whey protein isolate (PROLACTA-90; Lactalis Industrie, Bourgbarré, France) containing 11.95 g of leucine/100 g of amino acid. Carbohydrate was composed of maltodextrin (Glucidex IT21W; Sugro AG, Basel, Switzerland) and fructose (Fructofin C; Danisco, Kotka, Finland) at a ratio of 2:1, and fat was palm oil powder (Palmstearin 54; JuChem Food Ingredients GmbH, Eppelborn, Germany). Both supplements also contained 490 ± 51 mg of sucrose ester emulsifier (Ryoto S-1670; Mitsubishi Chemical Europe GmbH, Düsseldorf, Germany), 300 ± 31 mg of aroma (CHOC505176; Firmenich SA, Meyrin, Switzerland), and 630 ± 65 mg of NaCl per serving. To minimize disturbance to the plasma pool steady-state enrichment of 1-^{13}C-leucine during day 1 infusion, the LEUPRO supplement contained ~8% 1-^{13}C-leucine added during drink preparation.

LEUPRO supplementation was designed to saturate post-exercise muscle protein synthesis, suggested at the time of design conception to be at a leucine intake of ~0.135 g·kg^{-1} (~10 g·h^{-1}) (7) and 6–20 g of EAA (24,38). LEUPRO supplementation provided CHO at 1.2 g·kg^{-1}·h^{-1} to saturate glycogen synthesis (6), and moderate lipid was used for successful blinding between treatments and to aid in restoration of intramuscular lipid stores.

Whole-body leucine and glucose turnover. Whole-body leucine turnover was measured using a primed constant 1-^{13}C-leucine infusion during the 3-h postexercise recovery period on day 1 and at rest (preexercise) and during steady-state exercise on day 6. On day 1, the participants provided a urine sample and body mass measurement. A 20-gauge catheter was positioned in an antecubital vein of each arm; to the first catheter, an extension line leading to an infusion pump (74900 Series; Cole-Parmer Instrument Co., Vernon Hills, IL) was attached, whereas the second catheter was kept patent with 0.9% isotonic saline and closed with a two-way valve. After priming doses of 0.295 mg·kg^{-1} NaH^{13}CO$_3$ and 1 mg·kg^{-1} 1-^{13}C-leucine, the cyclists underwent a 4.5-h constant infusion of 1 mg·kg^{-1}·h^{-1} 1-^{13}C-leucine starting at 90 min into exercise. Blood samples for α-[^{13}C]-ketoisocaproate acid (α-KIC) enrichment and amino acid concentrations were taken preexercise, before infusion priming, after exercise immediately before supplement ingestion (defined as 0 min, the start of recovery), and at 30, 60, 90, 120, and 180 min into recovery.

On day 6, fasted participants provided body mass and urine, and catheters were fitted as described. At rest, participants received priming doses of NaH^{13}CO$_3$, 1-^{13}C-leucine, and 6,6-^{2}H$_2$-glucose (3.1 μg·kg^{-1}) before beginning constant infusions of 1-^{13}C-leucine and 6,6-^{2}H$_2$-glucose (initially at 40 μg·kg^{-1}·min^{-1}) for 180 min. At the onset of exercise, the 6,6-^{2}H$_2$-glucose infusion rate was increased stepwise at 0, 5, and 10 min to 60, 80, and 100 μg·kg^{-1}·min^{-1} (8) where it continued for the duration of the 90-min steady-state exercise. Blood was taken before infusion, preexercise, and at 60, 75, and 90 min into exercise. Blood samples for α-KIC and 6,6-^{2}H$_2$-glucose concentrations and enrichments were collected into EDTA and lithium heparin-treated evacuated tubes (Becton-Dickson, Auckland, New Zealand), respectively, and samples were collected and stored at −80°C until analysis. All infusates were 99 at.% and sourced from Cambridge Isotopes (Andover, MA).

Breath collection. Expired breath samples for indirect calorimetry were directed through a 5-L mixing chamber attached to a Douglas bag for 10- to 12-min gas collections at rest and 3–5 min during exercise. From a port on the mixing chamber, 3 × 10-mL samples were collected into screw-capped glass vials (Exetainer; Labco Ltd., High Wycombe, United Kingdom) for breath ^{13}C enrichment. On day 1, samples from Exetainer vials were collected during exercise before 1-^{13}C-leucine infusion (background), and at recovery times 0, 30, 60, 90, 120, and 180 min. On days 4 and 6, samples from Exetainer vials were collected at rest and at 60, 75, and 90 min into steady-state exercise.

Additional blood collection. Blood samples for additional parameters were collected on days 1 and 6 at sampling times as described for leucine/glucose infusions, as well as after exercise on day 6; preexercise and postexercise samples were taken on days 2 and 4 by venipuncture. Whole blood was collected into chilled EDTA vacutainers and centrifuged (15 min at 1750*g*, 4°C) for plasma CK and glucose; blood for insulin sat for 30 min in a serum vacutainer and was then centrifuged (30 min at 2000*g*). Perchloric acid extraction of plasma for amino acid analysis was performed as described by Moore et al. (25). For the analysis of plasma metabolites, 3 mL of whole blood sat for 3 min away from light in an ice-cold vacutainer and was then spun (2500*g*, 4°C for 10 min). For all parameters, a sample of supernatant was extracted to Eppendorf tubes and immediately frozen at −80°C until analysis.

Urine and sweat collection. Throughout the 6-d block, 24-h urine was collected for quantification of nitrogen excretion and urinary metabolite analysis (Fig. 1). From these data, the protein oxidation component to $\dot{V}O_2$ and $\dot{V}CO_2$ was calculated and corrected (18). Sweat was collected during exercise on day 2 and during the 90-min steady-state ride on day 6 using 7.5-cm^2 gauze pad set on parafilm within an adhesive patch and applied to two chest regions (superior to the nipple and ~5 cm lateral to the sternum) and to

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two abdominal regions (midpoint between the costal border and iliac crest, ~5 cm lateral to the sternum). After exercise, patches were removed, and samples were stored and frozen (−80°C) until analysis. Participants were asked to toilet, towel dry, and take nude bodyweight before and after collection periods for weight change sweat loss calculations.

Analyses

Isotopic enrichments, leucine kinetics, and glucose R_a, R_d, and metabolic clearance rate. Plasma enrichment of the *t*-butyldimethylsilyl derivative of α-KIC was measured by gas chromatography–mass spectrometry (GC–MS; Hewlett-Packard 6890; MSD model 5973 Network; Agilent Technologies, Santa Clara, CA). Calculations of leucine turnover were based on the reciprocal pool model where α-KIC enrichments were used as a proxy for plasma leucine enrichment, using equations described previously (23). A bicarbonate retention factor of 0.81 at rest and 1.00 during exercise was used (12). Calculations included a correction of 33% for first pass splanchnic extraction of 1-^{13}C-leucine (4) ingested with LEUPRO supplementation. However, because of the quantity of leucine ingested during feed recovery on day 1, the rate of uptake of free 1-^{13}C-leucine oral tracer was unknown. Given the quantities of leucine, protein, and CHO delivered, the rate of breakdown was most likely to decrease during recovery (4,17); conservatively, we have assumed a constant rate of breakdown throughout recovery with the LEUPRO supplement that is equal to the resting sample before supplement ingestion (where $B = Q - i$), and this clamped breakdown rate was used for subsequent calculations. Standard equations (i.e., without a clamped breakdown rate) apply for the control on day 1 (i.e., $I = 0$) and for day 6 (fasted) infusions for both conditions (23).

Isotopic enrichment of glucose was determined using GC–MS of the trimethylsilyl derivative and Steele equations modified for stable isotopes to calculate glucose R_a, R_d, and metabolic clearance rate using a volume of distribution of glucose of 100 mL·kg^{-1}, as described by Phillips et al. (29). Selected ion mass-to-charge ratio (*m*/*z*) was recorded at 205 and 207 amu. Glucose R_a and R_d were calculated at rest and at 60, 75, and 90 min during exercise and averaged throughout.

Plasma amino acid, glucose, and insulin concentrations. Plasma amino acid concentrations were measured from the perchloric acid extract by high-performance liquid chromatography (25). Glucose was assayed by spectrophotometric measurement using an automated analyzer (Roche/Hitachi 917; Roche, Auckland, New Zealand) of enzyme-catalyzed nicotinamide adenine dinucleotide phosphate formation from glucose, using an *in vitro* test kit (Roche/Hitachi Glucose Kit; Roche). Insulin was assayed via a standard immunoassay kit (Insulin ELISA IS130D 96 Tests; Calbiotech, Inc., Spring Valley, CA).

Muscle membrane damage. Serum CK activity was measured spectrophotometrically using a kit (Roche/Hitachi Total Creatine Kinase Kit; Roche).

Urea and creatinine. Net nitrogen balance was calculated during five ~24-h collections (day 1 being only ~12 h) to a total of 108 h (Fig. 1). Nitrogen intake was calculated from the protein content of each dietary food item, divided by an appropriate amino acid–nitrogen, based on Australian Food Composition Tables (NUTTAB 2006). Measured nitrogen outputs were urinary urea (Thermo Electron Urea Assay; Thermo Fisher Scientific, Inc., Waltham, MA) and creatinine (Creatinine Kit; Roche), with additional estimated nitrogen losses from sweat at rest and during exercise on days 2 and 6 and from feces and other miscellaneous losses throughout the collection period based on the urine with sweat loss ratios reported previously (36).

Metabolomic analysis of plasma and urine. Steps for the preparation of plasma and urine samples and for the preparation of kit standards are described in Supplementary Digital Content 2, http://links.lww.com/MSS/A110. Plasma samples were analyzed using an AbsoluteIDQ kit (Biocrates Life Sciences AG, Innsbruck, Austria) and liquid chromatography tandem mass spectrometry. Twenty-microliter samples were injected into the system (UltiMate 3000; Dionex AG, Olten, Switzerland) directed to the 3200 Q TRAP tandem mass spectrometer instrument equipped with a TurboIonSpray ionization source (AB Sciex; Foster City, CA). MetIQ software (Biocrates Life Sciences AG) was used to calculate metabolite concentrations and quality assessment. Urine samples were analyzed using EZ:Faast amino acid kits (Phenomenex, Inc., Torrance, CA) and GC–MS (Agilent 6890 GC coupled to Hewlett Packard 5975 MS; Agilent Technologies Schweiz AG, Basal, Switzerland). Samples were transferred into an insert within sample vials for analysis by GC–MS using GC conditions set to kit guidelines. Ions measured in GC–MS selective ion monitoring mode are described in Supplementary Digital Content 2, http://links.lww.com/MSS/A110. All samples were prepared and run in duplicate; coefficient of variation (CV) tolerance was set at 15%.

Statistical Analysis

Sample size calculation. Sample size was generated based on sufficient power to declare the effect of treatment on performance was likely greater than the defined smallest worthwhile effect, when using inference by magnitude-based precision of estimation as described by Hopkins et al. (16). The smallest worthwhile effect of treatment on performance is between 0.3 and 0.7 times the estimate for the CV (15). The relationship between performance in our repeat-sprint test (CV = 3.1%) (34) and competition performance has not been established, but the CV is within the range of other estimates for repeated-sprint tests and cycling competition (37). Powered to detect 0.5CV yielded $n = 24$; however, the magnitude of our anticipated effect was 4.1% (32), reducing n to 10. Two-thirds through data collection, we added two further subjects to correct for a randomization error in two cyclists to retain effective power within the crossover.

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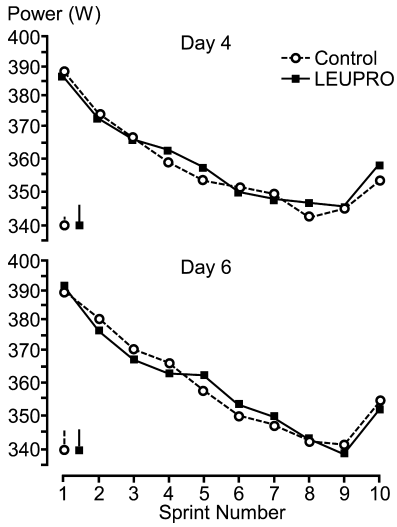

FIGURE 2—Sprint mean power during the performance tests on days 4 and 6. Data are back log-transformed least-squares mean concentrations plotted on a log scale with composite between-subject SDs obtained from the analysis.

Analysis. The effect of treatment on outcomes was estimated with mixed modeling (Proc Mixed, SAS Version 9.1; SAS Institute, Cary, NC). Most outcome variables were 100 × log-transformed before modeling to reduce nonuniformity of error and to express outcomes as percentages (16), with the exception of data sets with negative values (nitrogen balance). Most outcomes and comparisons were generated from fixed-effects models based on the interaction between the respective levels of treatment, test day, and order of treatment. For the analysis of sprint mean power, sprint number was a numeric effect (as in linear regression). Appropriate random-effect models for each parameter included all or some of between-athlete variation, additional treatment-associated variation, and additional variation associated with moving between test days. Variability between blocks at baseline in blood measures was identified *a priori* as a potential confounder; value at baseline was hence included as a covariate within the appropriate model. All covariates were first normalized to and expressed as a proportion of the within-subject SD for the covariate. In keeping with trends in inferential statistics, we used the magnitude-based approach to inferences as recently described (16,32). For performance, we used 0.93% (0.3 × 3.1%) as the threshold for small to align inferentially with our recent work (32,37), whereas for a mechanistic outcome, we used the standardized difference (effect size, ES) (16,32).

RESULTS

Performance. The effect of LEUPRO on sprint mean power was trivial on day 4 (0.4% ± 1.0%, $P = 0.51$) and day 6 (−0.3% ± 1.0%, $P = 0.63$) (Fig. 2); effects on fatigue (slope) on both days 4 (0.9% ± 5.4%, $P = 0.68$) and 6 (0.6% ± 3.4%, $P = 0.77$) were inconclusive. The likelihoods that the observed mean effect of LEUPRO was substantially detrimental/trivial/beneficial were 1.4%/79.6%/19% on day 4 and 2.1%/83.4%/14.5% on day 6.

Whole-body leucine and glucose kinetics. During recovery on day 1, there were very large increases in whole-body leucine flux, oxidation, and nonoxidative disposal with LEUPRO supplementation, relative to the control, whereas whole-body net leucine balance was positive with LEUPRO but negative for control (Table 1 and Fig. 3). At rest on day 6, the effect of LEUPRO supplementation on leucine flux, oxidation, nonoxidative disposal, breakdown, and balance was trivial (Table 1). Exercise increased the rate of whole-body leucine oxidation 1.6-fold (90% confidence limits (CL) = ×/÷1.1) and 1.9-fold (×/÷1.1) with LEUPRO and control, respectively, relative to rest. Whole-body leucine nonoxidative

TABLE 1. Effect of LEUPRO relative to control supplementation on whole-body leucine kinetics during recovery from exercise on day 1 and during rest and exercise on day 6.

Period	Parameter	Mean Effect (fold)[a]; ×/÷90% CL[b]	Effect Size[c]; ±90% CL[b]	*P*	Inference[d]
Day 1 recovery (60–180 min after exercise)					
	Q	5.20; ×/÷1.10	14.6; ±5.1	6e − 14	Almost certain
	OX	5.62; ×/÷1.11	4.28; ±1.33	9e − 16	Almost certain
	B	0.76; ×/÷1.09	−0.77; ±0.44	26e − 16	Almost certain
	NOLD	4.79; ×/÷1.13	15.39; ±5.43	3e − 12	Almost certain
	BALANCE[a]	504; ±87	17.8; ±5.8	3e − 9	Almost certain
Day 6 rest (preexercise)					
	Q	1.02; ×/÷1.14	0.07; ±0.38	0.74	Unclear
	OX	1.08; ×/÷1.12	0.19; ±0.27	0.20	Possible
	B	1.02; ×/÷1.14	0.07; ±0.37	0.75	Unclear
	NOLD	1.01; ×/÷1.16	0.04; ±0.43	0.88	Unclear
	BALANCE[a]	−2.0; ±7.5	0.18; ±0.67	0.66	Likely
Day 6 exercise					
	Q	1.05; ×/÷1.11	0.28; ±0.57	0.37	Unclear
	OX	0.90; ×/÷1.16	−0.38; ±0.58	0.25	Possible
	B	1.06; ×/÷1.12	0.28; ±0.57	0.39	Unclear
	NOLD	1.14; ×/÷1.13	0.50; ±0.48	67e − 3	Likely
	BALANCE[a]	2.7; ±5.6	−0.18; ±0.38	0.42	Unclear

[a] Data for Q, OX, B, and NOLD are the fold difference for the LEUPRO condition minus control. BALANCE is the absolute difference (μmol·kg^{-1}·h^{-1}).
[b] Multiply and divide this number by the mean effect to obtain the upper and lower CLs. For BALANCE, add and subtract.
[c] Effect size thresholds: <0.2, trivial; substantial outcomes: <0.6, small; <1.2, moderate; <2.0, large; <4.0, very large; >4.0, extremely large (14).
[d] Thresholds for assigning a qualitative inference to the likelihood of a substantial outcome: <1.0%, almost certainly not; <5.0%, very unlikely; <25%, unlikely; <75%, possible; >75%, likely; >95%, very likely; >99%, almost certain; an effect is unclear if its confidence interval includes both substantial increases and decreases (14).
B, leucine breakdown; BALANCE, whole-body net leucine balance; NOLD, nonoxidative leucine disposal; OX, leucine oxidation; Q, leucine flux.

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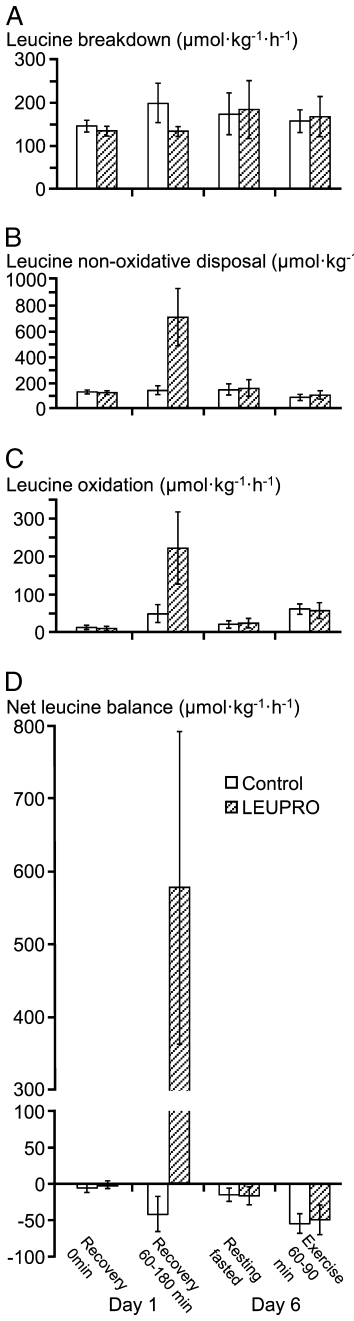

FIGURE 3—Whole-body leucine kinetics. Whole-body leucine breakdown (A), nonoxidative disposal (B), oxidation (C), and balance (D) immediately after exercise (recovery 0 min) and during recovery (60–180 min) on day 1 and at rest and during steady-state exercise on day 6. Data are means ± SD.

disposal was greater during exercise with LEUPRO (Table 1 and Fig. 3). Leucine enrichments are shown in Supplementary Digital Content 3, http://links.lww.com/MSS/A112.

At rest on day 6, there was a possible small increase in glucose R_a with LEUPRO of 3.1% (±3.6%, P = 0.14) relative to control, but the increase in R_d 1.7% (±6.2%, P = 0.62) was trivial at (R_a: LEUPRO = 14.2 ± 1.0 μmol·kg^{-1}·min^{-1}, control = 13.8 ± 1.1 μmol·kg^{-1}·min^{-1}; R_d: LEUPRO = 14.2 ± 1.5 μmol·kg^{-1}·min^{-1}, control = 13.9 ± 1.1 μmol·kg^{-1}·min^{-1}). During exercise on day 6, the effect of LEUPRO on glucose R_a was likely trivial (0.8% ± 3.0%, P = 0.63); outcomes were otherwise inconclusive (R_a: LEUPRO = 16.9 ± 1.0 μmol·kg^{-1}·min^{-1}, control = 16.8 ± 1.1 μmol·kg^{-1}·min^{-1}; R_d: LEUPRO = 17.7 ± 9.0 μmol·kg^{-1}·min^{-1}, control = 17.0 ± 9.0 μmol·kg^{-1}·min^{-1}).

RER. There were no clear effects of treatment on the RER (range of means = 0.86–0.90) during recovery from exercise on day 1 and at rest or during exercise on days 4 and 6 (data not shown).

Nitrogen balance. Nitrogen balance was positive on all of the five 24-h collection periods with the LEUPRO supplement; with control, nitrogen balance was negative from the completion of exercise on day 1 through to the morning of day 2 (Fig. 4A). The net difference in nitrogen balance between LEUPRO and control on day 1 was very large (ES = 2.4, 90% CL = ±0.60, P = 8e − 5; Fig. 4B); differences in 24-h nitrogen balance between LEUPRO and the control were otherwise inconclusive on days 2 and 3 and almost certainly trivial on days 4 and 5 (Fig. 4B). During the entire 6-d block (days 1–5), there was large net nitrogen gain with LEUPRO (ES = 0.95 ± 0.48, P = 4e − 03) but no clear gain with control (ES = 0.15 ± 0.52, P = 0.61), with a very large positive treatment differential (ES = 2.8 ± 1.6, P = 2e − 12); however, when day 1 nitrogen balance was excluded (i.e., during days 2–5), there was a large net nitrogen gain for both LEUPRO (ES = 1.03 ± 0.56, P = 7e − 03) and control

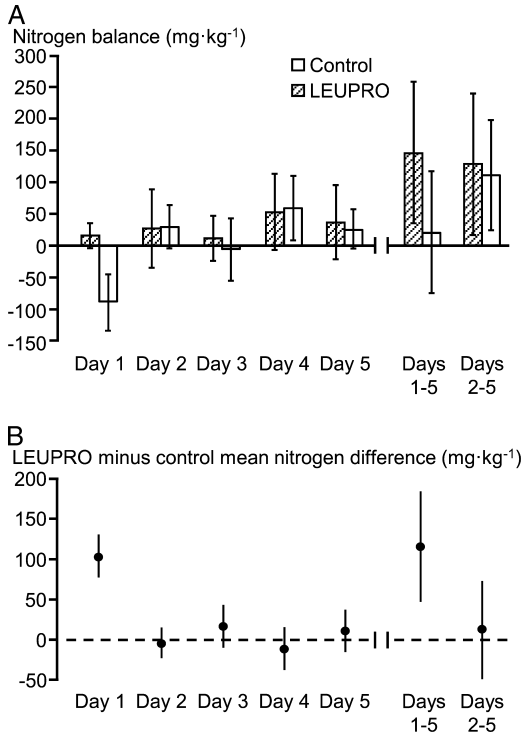

FIGURE 4—Effect of LEUPRO and control supplementation on nitrogen balance. Shown are mean ± SD daily nitrogen balance for collection periods on day 1 (~12 h) and on days 2, 3, 4, and 5 (all 24 h) and total nitrogen balance during days 1–5 and days 2–5 (A), and the difference between treatments by collection period with the 90% confidence interval (B).

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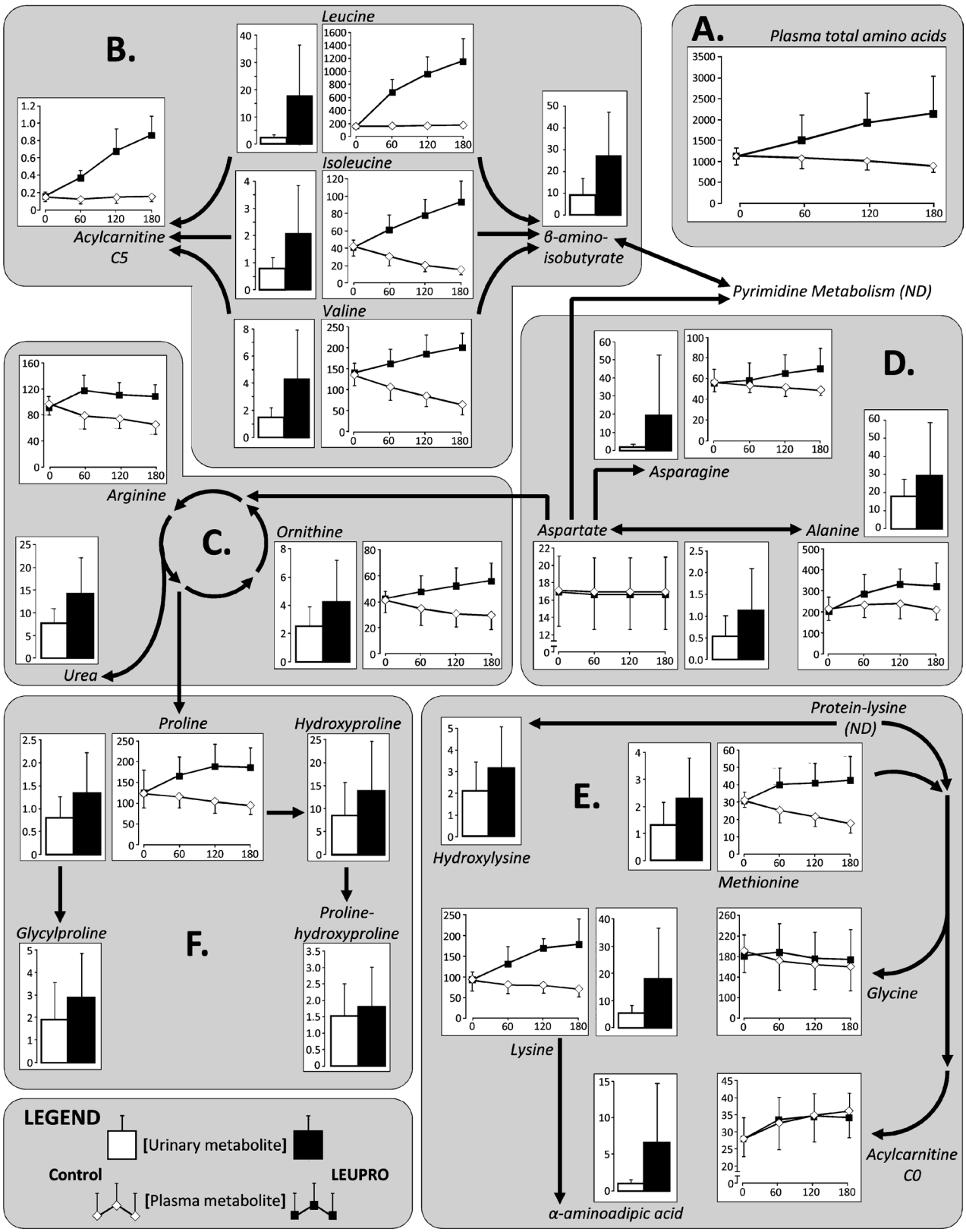

FIGURE 5—Plasma and urinary metabolite concentrations during recovery from exercise on day 1. Plasma essential and total amino acid concentration (A), and plasma and urinary concentrations of substrates and metabolites relating to the branched-chain amino acids (B), the urea cycle (C), the metabolism of alanine and aspartate (D), the degradation of lysine (E), or the metabolism of arginine and proline (F) during recovery on day 1. Data are means ± SD. Concentrations of plasma metabolites are in micromoles per liter ($\mu mol \cdot L^{-1}$) and those of urinary metabolites are in nanomoles per liter ($nmol \cdot L^{-1}$). ND, not determined.

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(ES = 0.90 ± 0.52, P = 0.01), and the treatment differential was unclear (ES = 0.15 ± 0.70, P = 0.73; Fig. 4B).

Plasma concentrations of glucose and insulin. Overall mean plasma glucose and insulin concentrations during the 3-h recovery period after exercise on day 1 in the control were 6.9 ± 1.6 mmol·L^{-1} and 89 ± 118 μIU·mL^{-1}.; the LEUPRO supplement led to a 12% reduction (±7%; ES = −0.80 ± 0.51, P = 7e − 3) and a possible increase of 17% (±34%; ES = 19 ± 34, P = 0.23), respectively (data not shown for brevity).

Metabolomics. The effect of supplementation on the concentrations of urinary and plasma metabolites during 3 h of recovery on day 1 is shown in Figure 5; nomenclature and the full listing of mean effect sizes and other statistics for the treatment differential can be found in Supplementary Digital Content 4, http://links.lww.com/MSS/A113. On day 1 in the control, the total plasma amino acid concentration declined with time from the start of recovery (Fig. 5A). However, LEUPRO supplementation led to an extremely large increase in plasma leucine (3.5-fold; 90% CL = ×/÷1.1) and a very large increase in essential (2.2-fold; 90% CL = ×/÷1.1) and total amino acid (1.7-fold; 90% CL = ×/÷1.1) concentrations, relative to the control (Figs. 5A and B). LEUPRO supplementation also resulted in small, moderate, and large increases in the plasma concentration of acylcarnitines related to BCAA metabolism, C4 (1.2-fold; 90% CL = ×/÷1.2), C3 (1.5-fold; 90% CL = ×/÷1.1), and C5 (3.0-fold; 90% CL = ×/÷1.1), respectively. During exercise on day 6, LEUPRO caused small increases in plasma acylcarnitines C3 (1.1-fold; 90% CL = ×/÷1.1) and C5-M-DC (1.2-fold; 90% CL = ×/÷1.3) and a moderate increase in C2 (1.3-fold; 90% CL = ×/÷1.3), but a small decrease in C16 (0.92-fold; 90% CL = ×/÷1.2), relative to the control. The effect of LEUPRO supplementation on day 6 plasma amino acid concentrations and other plasma acylcarnitines, glycerophospholipids, and sphingolipids was mostly trivial or inconclusive (Supplementary Digital Content 4, http://links.lww.com/MSS/A113).

LEUPRO ingestion during recovery on day 1 also resulted in small to extremely large increases in the urinary concentrations of products of the metabolism of branched-chain amino acids, alanine and aspartate, arginine and proline, lysine, and urea cycle metabolites (Figs. 5B–F), as well as cysteine and methionine, phenylalanine and tyrosine, and tryptophan, relative to control ingestion (Supplementary Digital Content 4, http://links.lww.com/MSS/A113). During exercise on day 2, there were likely moderate reductions in proline (−18% ± 18%), methionine (−19% ± 19%), isoleucine (−20% ± 18%), and α-aminobutyric acid (−17% ± 17%) and a large reduction in asparagine (−19% ± 19%) with LEUPRO compared to the control; outcomes for other urinary metabolites during day 2 exercise and on days 3, 4, and 5 were mostly inconclusive or trivial (Supplementary Digital Content 4, http://links.lww.com/MSS/A113).

At rest on day 6, LEUPRO produced moderate reductions in urinary leucine (−26% ± 19%), isoleucine (−23% ± 17%), and valine (−36% ± 22%) concentrations and small to moderate reductions in products of the metabolism of proline, cysteine and methionine, phenylalanine and tyrosine, and the tryptophan metabolite kynurenate, relative to control supplementation (Supplementary Digital Content 4, http://links.lww.com/MSS/A113). This effect was reversed during exercise on day 6, with small increases in the urinary excretion of leucine (27% ± 30%) and isoleucine (26% ± 26%) and small to moderate increases in methionine, phenylalanine and tyrosine, proline, tryptophan, and products of their metabolism (Supplementary Digital Content 4, http://links.lww.com/MSS/A113).

CK. Before and after exercise, mean ± SD plasma CK concentration (U·L^{-1}) in the control condition was as follows: day 4 = 170 ± 88 and 210 ± 94; day 6 = 145 ± 63 and 195 ± 72. Relative to the control values, LEUPRO led to small to moderate reductions before (24%–25% ± 14%, ES = −0.66 to −0.70 ± 0.33, P = 4e − 3 to 7e − 3) and after (21%–22% ± 14%, ES = −0.59 to −0.61 ± 0.33, P = 0.01 to 0.02) exercise on days 4 and 6.

DISCUSSION

Postexercise supplementation with LEUPRO increased the rate of whole-body nonoxidative leucine disposal and resulted in positive leucine and nitrogen balance during the immediate hours after an intense endurance exercise. The supplement also attenuated the increase in CK during the 6-d cycling block, suggesting lower tissue disruption. These outcomes, however, were associated with trivial effect on subsequent high-intensity cycling performance, which might have been influenced by positive mean daily nitrogen balance. Furthermore, LEUPRO ingestion during recovery increased the concentration of plasma and urinary metabolic intermediates of BCAA degradation, indicative of protein and leucine intake that exceeded the whole-body capacity to metabolize BCAA.

Association among protein–leucine feeding, nitrogen balance, and performance. We reported previously that high protein–CHO or leucine-enriched protein–CHO postexercise feeding can improve subsequent repeated-sprint performance (32,37). Therefore, the likely trivial effect of LEUPRO on performance was somewhat unexpected. Our recent work indicated that the observed enhancements to repeated-sprint mean power were small (2.5%, 99% CL = ±2.6% [37]) to moderate (4.1%, 95% CL = ±4.1% [32]), with uncertainty allowing for very large to trivial performance outcomes. Given the overlap in confidence intervals across all three investigations in this series of work, it is possible that sampling variation could account for mean differences in subsequent performance between studies. On the other hand, several design and outcome related factors could also explain the unremarkable performance effect and yield new insight into the physiological responses resulting from postexercise ingestion of leucine-enriched protein–CHO that could affect recovery processes and subsequent performance.

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First, postexercise high-protein feeding during a period of intense endurance training might not substantially benefit subsequent performance (relative to nil or low-protein isocaloric control) if nitrogen balance is positive. Rowlands et al. (32) reported that low-protein high-CHO feeding in the 4 h after exercise totaling 0.92 g protein·kg^{-1}·d^{-1} (including controlled diet at other times) was insufficient to meet nitrogen requirements when compared with high protein–CHO feeding that provided 2.97 g protein·kg^{-1}·d^{-1}. To our knowledge, this was the first study to suggest that nitrogen balance status might be linked to performance. However, from the design (in which daily protein intake was not balanced), it was not possible to establish if improved performance was the result of positive daily nitrogen balance in the intervention versus negative in control or to the postexercise protein feeding. In the following study that provided further insight into the possible nitrogen balance performance association, Thomson et al. (37) clamped dietary protein intake at 1.6 g·kg^{-1}·d^{-1} during the experimental block but observed in both protein–leucine and control postexercise feeding conditions a mild average nitrogen deficiency, equivalent to 0.12 and 0.14 g protein·kg^{-1}·d^{-1}, respectively. This latter study showed that, during a 5-d block of intense training, protein–leucine supplemental feeding for 1.5 h immediately after exercise can benefit subsequent performance when nitrogen balance is mildly negative. To our surprise, in the current study, overall nitrogen balance was positive in both conditions during days 2–5 of the 6-d protocol (Fig. 4). Positive nitrogen balance may be assumed to represent net protein gain, which would likely reflect changes in metabolic processes, such as an increased postprandial protein synthesis rate or reduced protein breakdown (10). Therefore, sufficient tissue anabolism may have occurred in the control condition to have negated the relative positive protein synthetic and anticatabolic effects of protein–leucine feeding. Taken together, our collective data suggest that a net relative negative nitrogen balance might be required for postexercise high-protein–CHO or leucine-enriched protein–CHO feeding to benefit subsequent performance. Nevertheless, postexercise protein–leucine ingestion still lowered plasma CK concentrations, which suggests that attenuation of membrane disruption may be insensitive to nitrogen balance.

Second, if protein intake during the experimental period differed considerably from the habitual intake, a period of adaptation would be required that could have affected nitrogen balance (31) and may have contributed to differences in nitrogen balance between the current study and that of Thomson et al. (37). Although the protein composition of the lead-in diet was not determined in the current study, substantially positive nitrogen balance in the control condition during days 2–5 could indicate that protein intake was greater with the experimental diet than with the habitual diet. Prestudy diet diaries collected by Thomson et al. (37) estimate mean habitual protein intake of 2.0 g kg^{-1}·d^{-1} by their cohort, which might explain their findings of negative nitrogen balance. Given that our cohort was extremely similar (and, therefore, likely consume similar diets), an alternative explanation to account for our findings of positive nitrogen balance could be that the experimental diet in the current study provided a greater quantity of non–vegetable-based proteins and a more even distribution of protein across the daily diet. Nitrogen retention from animal-derived proteins seems to be superior (11) and consuming the majority of dietary protein at the opposite end of the day to exercise (Thomson et al. [37]) would limit the effect of dietary protein on postexercise muscle protein synthesis versus dietary protein consumed evenly across the day, as in the current study. Further research intervening on chronic nitrogen balance with control of other covariates is warranted to explore the hypothesis that nitrogen balance has an important role in determining the efficacy of postexercise high-protein–CHO feeding on skeletal muscle protein metabolism, recovery processes, and endurance performance.

Effects of protein–leucine feeding on leucine turnover during recovery. It has been suggested that the primary outcome of protein ingestion for endurance athletes should be to replace exercise-associated oxidative protein losses and support amino acid requiring metabolic processes, especially an increased rate of postexercise protein synthesis (30). Net muscle protein balance and fractional muscle protein synthesis after exercise is associated with extracellular essential amino acid concentration (5,26). Without protein ingestion (control supplementation) during recovery, the plasma essential, leucine, and total amino acid concentration decreased with time, whereas whole-body leucine oxidation was increased, indicating that amino acid availability for metabolic processes could have become limiting. However, LEUPRO ingestion increased recovery plasma amino acid concentrations and established net positive whole-body leucine balance (Fig. 3). Furthermore, the increased plasma amino acid availability would most likely have been sufficient to offset the elevated rate of whole-body protein oxidative losses and support the increased tissue protein synthesis rate.

Based on the large increases in postexercise whole-body leucine kinetics with LEUPRO supplementation, and the findings of Howarth et al. (17) and others (21,24,38), it is likely that LEUPRO ingestion increased the postexercise muscle protein synthesis rate in the immediate few hours of recovery, relative to the control. However, we acknowledge that whole-body protein kinetics might not necessarily reflect changes in skeletal muscle protein turnover (17,21) and that expansion of the intracellular α-KIC pool could explain some of the changes in leucine kinetics. Furthermore, we note that there is some discrepancy in whole-body leucine turnover findings among similar postendurance exercise protein–CHO feeding studies in trained men using the 1-^{13}C-leucine infusion method. Levenhagen et al. (22) reported only a 15% increase in nonoxidative leucine disposal with protein–CHO feeding (8/5/3 g protein/CHO/fat) after 1 h of cycling at 60% of $\dot{V}O_{2max}$, relative to protein-free and placebo conditions. The different magnitude of the effect relative to the current

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study (~380% increase in nonoxidative leucine disposal) is most likely because Levenhagen et al. used only 8 g of protein, which Moore et al. (26) have shown would not saturate mixed-muscle FSR postresistance exercise. Comparably, the recent investigation by Howarth et al. (17) is more like the present in terms of exercise undertaken and postexercise nutrition. The authors reported that the addition of protein to CHO led to increased postexercise leucine oxidation (4-fold vs 5.6-fold in the current study) and positive whole-body net leucine balance, but most importantly, that mixed-muscle FSR was increased by 50% (17). However, nonoxidative leucine disposal was not significantly different (17). It might be that the added leucine in the LEUPRO condition could explain our present observation of increased nonoxidative leucine disposal. Leucine potently stimulates tissue protein synthesis in the rodent model (7) and, when added to protein and CHO ingested after resistance exercise in men, leads to a small increase in the already-positive whole-body net protein balance and moderately increased rate of mixed-muscle FSR (21). Nevertheless, as we have no comparison condition, we cannot differentiate the effect of leucine (or protein) alone. Overall, it is reasonable to suggest that LEUPRO ingestion increased the muscle protein synthesis rate in the hours after exercise, relative to the control. However, this did not transpire to a functional (performance) improvement during the 6-d block of intense cycling, suggesting that an increased muscle protein synthesis rate in the immediate hours after exercise is, by itself, not an important mechanism governing protein nutrition–related improvement in short-term (45–60 h) subsequent performance.

Amino acid metabolomics. Not surprisingly, LEUPRO supplementation resulted in large increases in the plasma and urinary concentrations of amino acids and products of their metabolism during recovery from exercise on day 1. Elevated plasma BCAA concentrations and leucine turnover with LEUPRO supplementation would almost certainly explain the increases in plasma acylcarnitines C3, C3:1, C4, C5, and urinary β-aminoisobutanoate on day 1. Conversely, the small increase in plasma acylcarnitine C3 during exercise on day 6 might instead be the result of a decline in the concentration of plasma palmitoylcarnitine, an inhibitor of C3 synthesis (3), or reduced metabolism to the C3 derivatives C3-OH and C3:1. Associated with the LEUPRO condition were reduced urinary losses of BCAAs, proline, methionine, and β-aminoisobutyrate (a product of both valine and pyrimidine metabolism) during exercise on day 2, suggesting reduced turnover or retention of these amino acids and metabolites. The more widespread alterations to the concentrations of plasma and urinary amino acids and their metabolites at rest and during exercise on day 6 (Supplementary Digital Content 4, http://links.lww.com/MSS/A113) could be the result of adaptive processes in response to higher intakes of leucine and protein associated with LEUPRO ingestion.

Recently, a mean upper metabolic limit to oxidize leucine in men (ages 18–35 yr) at rest was estimated at 556 $mg \cdot kg^{-1} \cdot d^{-1}$ (9). In comparison, LEUPRO supplementation during recovery provided leucine (free and protein bound) at a rate of ~130 $mg \cdot kg^{-1} \cdot h^{-1}$ for 3 h. Increased concentrations of plasma acylcarnitines C3 and C5 indicate that leucine ingestion of ~130 $mg \cdot kg^{-1} \cdot h^{-1}$ exceed the capacity of some enzymes involved in the metabolism of leucine after an acute bout of endurance exercise. Accumulation of amino acids and their secondary metabolites in the plasma and urine may indicate an upper tolerable limit of intake, above which might increase toxicity risk (28). However, we hypothesize that the increased whole-body leucine oxidation rate after endurance exercise might act to increase the upper tolerable limit and, therefore, postexercise supplementation with leucine (and protein) may be better tolerated than in resting individuals; adaptive alterations of amino acid metabolism to chronic high intakes could also alter leucine tolerance. Given recent interest in the role of postexercise protein ingestion to elevate and saturate postexercise whole-body and muscle protein synthesis (17,26), further work is needed to investigate metabolic responses to protein and leucine ingestion after exercise or with chronic feeding. This work should include sensitive and high-throughput mass spectrometry–based metabolomics to elucidate subtle but possibly large-scale perturbations to metabolism, which could represent important mechanisms of recovery or adaptation to exercise and protein feeding.

To conclude, ingesting a leucine-enriched protein supplement after high-intensity cycling resulted in a positive whole-body net leucine balance, reduced plasma CK, and led to the accumulation of plasma and urinary amino acids and their metabolites, during recovery from exercise. However, the supplement provided no clear benefit to subsequent performance, which contrasts previous findings by our group. We hypothesize that the likely trivial performance differential between the protein–leucine and control conditions was primarily the result of a positive nitrogen balance during the experimental period, ameliorating the benefit of postexercise protein–leucine feeding reported recently (37).

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The authors declare no conflicts of interest.

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The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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